Reprinted from Science, January 19, 1962, Vol. 135, No. 3499, pages 215–216

Food Production by Submerged Culture of Plant Tissue Cells

Abstract. In investigating the possibility of producing food by large-scale culture of plant tissue, several plant tissues were used. The yield for carrot tissue, which is especially capable of rapid growth, was an average of 4.6 grams per liter per day in 6 liters of medium. The results suggest that with improved techniques food could be produced by this method.

Within the past few years considerable progress has been made in the design of systems for accelerated food production. The goal of much of this work is to establish the requirements for a practical method of feeding men under unusual survival conditions or, ultimately, during prolonged space travel.

Our work (*1*) was undertaken as an exploratory study to determine the feasibility of producing food by tissue-culture techniques. That large amounts of plant material may be obtained by growing specific tissues of higher plants in liquid media has been established (*2*). The large-scale culture of plant tissue for food, however, has not been previously studied, which is rather surprising in view of the potentials of a successful system of this type.

In seeking to discover the specific requirements for a large-scale food production system we used cultures of normal tissues of carrot and potato and of the stems of tomato, rose, grape, and tobacco (*3*). These tissues have been maintained on 0.6-percent agar "tobacco" medium at *p*H 5.7 to which coconut milk (15 percent by volume) and α-naphthaleneacetic acid (0.1 mg/lit.) were added. "Tobacco" medium without agar was used for the liquid cultures (*4*). All of the tissues grew well in submerged cultures, but, since the carrot tissue appeared to be especially capable of rapid growth, it was selected as the principal tissue for this study.

To scale-up liquid cultures to large volume we used Erlenmeyer flasks (300 ml), then Fernbach flasks (3 liters), and finally Florence flasks (12 liters). The inoculum for the largest flasks was prepared by transferring a piece of tissue from the agar to 100 ml of liquid medium in a 300-ml Erlenmeyer flask. This culture was agitated on a rotary shaking machine. When sufficient growth occurred (1 to 3 weeks), the contents were transferred to a Fernbach flask containing 1 liter of medium. This culture was also agitated on a shaker. After another period of growth, 200 to 300 ml of the culture were inoculated aseptically into 6 liters of medium in a 12-liter flask. The tip of a 100-ml volumetric pipette was cut off before sterilization to facilitate this transfer of tissue. To aerate and agitate the culture during growth, the flask was fitted with sterile tubing, by which compressed air, sterilized by passage through a 2-inch pipe packed with sterile cotton and two Seitz filter pads, could be injected into the flask. The pipe was connected to a compressed-air vent with a pressure regulator. Aseptic techniques were followed in all the procedures.

Table 1. Yields of carrot tissue from submerged cultures in 6 liters of broth.

Culture No.	Days cultured (No.)	Age of inoculum (days)	Yield (wet weight)		
			Total (g)	g/lit.	g/lit. day
1	6	7	167.1	27.8	4.6
2	6	19	160.4	26.7	4.4
3	7	35	167.2	27.8	3.9
4	5	2	183.5	30.6	6.1
5*	5	2	122	20.3	4.0

* Malt extract used in place of coconut milk.

At first, cultures grown in the large flasks were frequently contaminated with mold and bacteria. To control this contamination without inhibiting the growth of tissue, antibacterial Tylosin (20 parts per million) (*5*) and antifungal Mycostatin (25 units) (*6*) were added to the liquid medium before inoculation. These two antibiotics—now used routinely in our tissue cultures of carrot, grape stem, and Queen Elizabeth rose stem—have produced no toxic effects.

The cultures on the shaking machine were incubated at 28°C while the culture in the 12-liter flask was of necessity subjected to the variations of room temperature. It is assumed that a constant optimum temperature would increase the growth rate and the yields of tissue.

The yields of tissue from five large-volume cultures are shown in Table 1. Our method of large-volume culture did not provide for removing any of the tissue or for supplementing the medium. Consequently, the tissues were harvested after 5 to 7 days in culture because heavy growth almost stopped agitation by the aeration system.

Inocula from a young, actively growing culture contributed to an improved yield and decreased the lag phase of growth in the large flask (culture No. 4). Culture No. 5, with malt extract in place of coconut milk, was inoculated at the same time as No. 4 with material from the same Fernbach flask. By the use of a Y-tube the two 12-liter flasks were aerated simultaneously.

Our yields from 6 liters of medium averaged 4.6 g/lit. per day. They are slightly higher than the yields obtained by Tulecke and Nickell from cultures of *Gingko*, Holly, and *Lolium* tissue grown in 10 liters of medium in 20-liter carboys (3.1 g/lit. per day). Their pilot-plant tank cultures of rose stem gave a higher yield (9.7 g/lit. per day). However, they say this figure is subject to interpretation, since the culture period was only 2 days and a heavy inoculum was used in 134 liters of medium.

We feel that these yields are not indicative of the best results that could be obtained under better conditions of aeration and medium replenishment. It is conceivable that food could be produced by submerged culture techniques of plant tissue with a continuous cell generator having a capacity of ten or more liters and operating under optimal conditions for a long period of time.

ANNE F. BYRNE
ROBERT B. KOCH*
Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois

References and Notes

1. This paper reports research conducted by the Quartermaster Food and Container Institute for the Armed Forces and has been assigned Nr. 2114 in the series of papers approved for publication.
2. L. G. Nickell, *Proc. Natl. Acad. Sci. U.S.* **42**, 848 (1956); W. G. Tulecke and L. G. Nickell, *Science* **130**, 863 (1959); G. Melchers and U. Engelmann, *Naturwissenshaften* **42**, 564 (1955); W. G. Tulecke and L. G. Nickell, *Trans. N.Y. Acad. Sci.* **22**, 196 (1960).
3. We are indebted to Drs. A. J. Riker and A. C. Hildebrandt, Department of Plant Pathology, University of Wisconsin, for supplying us with the cultures of plant tissues.
4. A. C. Hildebrandt, A. J. Riker, B. M. Duggar, *Am. J. Botany* **33**, 591 (1946).
5. We thank the Lilly Research Laboratories, Eli Lilly and Co., for supplying us with Tylosin.
6. We express our thanks to E. R. Squibb and Sons for their generous gifts of Mycostatin.
* Present address: Honeywell Research Center, Minneapolis-Honeywell Regulator Co., Hopkins, Minn.

14 November 1961